The presence of Soprano pipistrelle *Pipistrellus pygmaeus* (Leach, 1825) in Switzerland: first molecular and bioacustic evidences

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The presence of Soprano pipistrelle *Pipistrellus pygmaeus* (Leach, 1825) in Switzerland: first molecular and bioacustic evidences. - The two widespread sibling species of pipistrelle bats, the soprano pipistrelle (*Pipistrellus pygmaeus*), and the common pipistrelle (*Pipistrellus pipistrellus*), that emit at distinct call frequencies (maximum energy at around 55 and 45 kHz respectively), have been found in sympatry over much of Europe, but little is known about their relative abundance and possible habitat use. In this study, we provide the first record of *P. pygmaeus* in southern Switzerland, confirmed by echolocation analysis and mitochondrial DNA sequencing. During 70 catching sessions in the southern region of the Swiss Alps, echolocating bat calls were recorded to define the distribution/presence of the two species. Habitat characteristics of capture sites suggested that the sibling species have a high degree of niche overlap (0.88). Nevertheless, *P. pygmaeus* seemed to select agricultural areas and riversides more frequently than *P. pipistrellus,* which is found more often in urban areas. Divergence in the cytochrome b gene of mitochondrial DNA of 37 bats confirmed the echolocation data for 31 of 33 *P. pipistrellus* and for two of four *P. pygmaeus,* while four animals, two of each species, had been misclassified based on echolocation only. A canonical discriminant function using morphological data was not reliable to distinguish the sibling species.

Key-words: Chiroptera - *Pipistrellus pygmaeus* - Sibling species - mtDNA - echolocation - Switzerland.

INTRODUCTION

The study of sibling species has great importance in community ecology, zoogeography and conservation. Sibling species exist in many zoological *taxa* (insects and Vertebrates as fishes, reptiles and rodents). Among European bats, this has been

demonstrated for *Myotis myotis* and *Myotis blythii* (Arlettaz *et al.*, 1993, 1997; Arlettaz, 1996) and for *P. pipistrellus* and *P. pygmaeus* (Barlow, 1997; Barlow & Jones, 1997, 1999). Populations of sibling bat species living in sympatry are defined as cryptic species, because they consist of individuals that are morphologically similar, or even identical, that can only be recognised by biomolecular or bioacoustical methods.

The case of the common pipistrelle bat (*Pipistrellus pipistrellus* Schreber, 1774) is probably one of the most surprising and interesting discoveries among European mammals: until 1999, this species was regarded as a single biological unit. In 1993 two different phonic types were discovered among British pipistrelles: individuals emitting two distinct search-phase echolocation calls were recognized, with frequencies of maximum energy at around 45 kHz and at around 55 kHz (Jones & Van Parijs, 1993). Subsequently, genetic analysis proved that the Europe's most widespread and well-studied bat actually exist as two cryptic species (Barratt *et al.*, 1997), separated by a sequence divergence of >11% in the cytochrome b gene of mitochondrial DNA. Despite the fact that a definitive taxonomic classification is not yet defined (von Helversen *et al.*, 2000; Mayer & von Helversen, 2001) we have chosen to adopt the name proposed by Jones & Barratt (1999), based on suggestions made by Hutson and Jones (von Helversen *et al.*, 2000: pp.115, 116). Jones and Barratt (1999) proposed the nomenclature *Pipistrellus pygmaeus* (Leach, 1825) for the phonic type "55 kHz" - popularly called soprano pipistrelle, thanks to its high-pitched call - while the phonic type "45 kHz" remains the common pipistrelle, *Pipistrellus pipistrellus*. The two phonic types can be readily identified by using a bat detector, and have been found in sympatry in Switzerland (Zingg, 1990; Letard & Tupinier, 1997), Great Britain (Jones & Van Parijs, 1993), North of Ireland (Russ, 1996), central Spain (Ruedi *et al.*, 1998; Mayer & von Helversen, 2001), Germany (Häussler *et al.*, 1999; Mayer & von Helversen, 2001), France (Lustrat, 1999) and in the South of Italy and Sardegna (Russo & Jones, 2000). In other countries the presence of the *P. pygmaeus* is recorded also for Greece (Weid & von Helversen, 1987; Mayer & von Helversen, 2001), Denmark, Norway and Portugal (Jones, 1997).

In the northern part of the Swiss Alps, a first bioacoustical record of two forms of echolocating common pipistrelles was made by Zingg already in 1990 even though Zingg didn't identify them as two different species but as two different phonotypes. The presence of two species, after the classification of Jones & Barratt (1999), has never been confirmed in Switzerland by biomolecular evidence. In this study, we investigate the occurrence of the two sibling species of *Pipistrellus* in the Southern region of the Swiss Alps, that includes the Cantone Ticino (Switzerland) and two northern provinces of Italy (Como and Varese), using biomolecular and bioacoustical analyses, and analysing environmental and morphological data.

MATERIALS AND METHODS

STUDY AREA AND CAPTURE METHODS

The study was carried out from May to October 2001. We visited 70 sites (Fig. 1), 52 in Cantone Ticino (Switzerland), 10 in the province of Como (Italy) and 8 in the province of Varese (Italy), where we captured different bat species. The study

FIG. 1

Study area. Dots indicate sampling sites.

area extends over 7152 km² (coordinates range: 46°33'46.8"N; 9°15'43.2"E; 45°35'16.8"S; 8°22'33.6"W) and the landscape consists of 20% lowlands, 45% hills and 35% mountains, with elevation ranging from 200 m a.s.l., at Lake Maggiore, Varese province, to over 3000 m in the Swiss Alps (Rheinwaldhorn, 3348 m), in the northern part of the study area. The continental climate is tempered by the large lakes, with relatively mild winters, and abundant precipitation ranging from 1500 to 2200 mm per year.

Most sites visited in the study area (n = 45) were maternity roosts located in houses that had been previously signalled by local people to the Centre for the Protection of Bats of the Canton Ticino, a local bat conservation group founded in 1990. A church, two factories and one cave were also checked for bats. Additionally, 19 foraging sites along rivers and water-courses (random points) and 115 bat boxes in two deciduous woodlands were monitored.

Bats were captured during evening emergence at roosts, using hand-nets, which allowed us to catch only part of the individuals in each of the colonies. Of all caught bats, morphological measurements were taken and their echolocation calls were recorded, while skin samples were taken from a maximum of five animals per site. The total number of animals emerging was counted.

At foraging sites, mist-nets were placed along ponds and water-courses, as described by Kunz (1988), that remained activated from dusk to midnight, and all the data described above were taken for each captured bat. Bats roosting in boxes, mainly males in mating groups, were taken by hand.

SAMPLING PROCEDURE

Each captured bat was sexed and aged. The age-class, (sub-adult or adult), was determined based on epyphyseal growth plates closure in the metacarpal-phalangeal joint of the fifth finger (Anthony, 1988). Reproductive condition was assessed check-ing testes development in males and lactation in females (Racey, 1988). Females were defined lactating when milk appeared while gently squeezing the nipples, or when the fur surrounding nipples was absent. Twelve morphological measurements of bats were taken using a precision callipers (± 0.01 mm): forearm length, wing span (from the wing extremity to the shoulder, gently stretching out the left wing,), III and V finger (from thumb insertion to III finger extremity and V finger extremity respectively, gently stretching out the left wing), II and III phalanx of III finger, thumb (from thumb inser-tion to thumb extremity without nail), foot (from heel to the third finger extremity with-out nail), tibia, tail (from anus to tail extremity), and ear length (from tragus insertion to ear extremity). Bats were weighed to the nearest 0.5 g with a 50 g Pesola dy-namometer. In a first step, bats were determined as belonging to one of the sibling species using their echolocation characteristics, morphological measurements were compared by multivariate analysis of variance (MANOVA, Sokal & Rohlf, 1995). In a second step, the morphological parameters that were significantly different between the two species were analyzed with the SYSTAT statistical package (SPSS, 1997) in order to calculate a Discriminant Function with Jackknife procedure (Hinkley, 1977) to determine the percentage of cases classified correctly (significance was tested with a F-transformed Wilk's lambda test, Tukey, 1977). Finally, a canonical discriminant function was calculated to distinguish between the two species.

For each bat, echolocation calls were recorded in time expansion mode with a Pettersson D980 bat detector, while releasing it from the hand in open habitat near the roost. Echolocation calls spectrograms were subsequently obtained (1024 points Fast Fourier Transform, Parzen window) and analysed using BatSound software (Pettersson, 1999). For statistical analysis, to avoid sample size problems, a subset of 18 randomly chosen recordings of *P. pipistrellus* were compared with the total data set

of 18 *P. pygmaeus*. For each recording, six characteristic frequency-domain parameters were measured: start frequency (F_{start}), maximum frequency (F_{max}), minimum frequency (F_{min}), frequency at maximum intensity (F_{maxint}), end frequency (F_{end}), and frequency at half the duration of the search phase call ($F_{t/2}$). Finally, call duration (in ms) was also measured. Each parameter was compared between the two sibling species with a non-parametric Mann-Whitney U-test. Significant parameters were then used in a Discriminant Function Analysis with Jackknife procedure, to obtain a classification function based on these echolocation parameters.

GENETIC ANALYSIS

Two tissue samples of 4 mm diameter were taken from the tail membrane (uropatagium) of each skin-sampled bat using a biopsy punch, and placed in a 0.2 ml eppendorf vial filled with 70% ethanol and stored at –20 °C until the moment of genetic sequencing. Genetic analyses were carried out on a total of 137 bats, for which also bioacoustic and morphologic data were available. All bats were handled with care and kept as little as possible to avoid stress, and no animals were injured or died during handling.

DNA from tissue samples was extracted using the QIAmp DNA Mini kit (Qiagen) following supplier's instructions. Each sample was amplified by PCR (Saiki *et al.*, 1988) using primers L14841 and H15149 (Kocher *et al.*, 1989), targeting a 307 base pairs (bp) portion of the mitochondrial DNA (mtDNA) cytochrome *b* (cyt *b*) gene. Double-stranded cycle sequencing was conducted using the ABI PRISM™ BigDye Terminator Cycle Sequencing Ready Reaction kit, Version 3.0 (Applied Biosystems). Purified sequencing products were loaded on a ABI PRISM™ 377 DNA sequencer (Applied Biosystems). Both strands have been sequenced with the same primers used in the PCR amplification. For species identification, the DNA sequences obtained from the samples were compared with homologous sequences of other Chiroptera available in DNA databases, in particular with the *Pipistrellus spp.* sequences from Barratt *et al.* (1997) (GenBank accession nr. U95499, U95501, U95503, U95505, U95507, U95509). Distance matrix calculation (Kimura 2-parameter distances; Kimura, 1980) and phylogenetic analysis (maximum parsimony, exhaustive search with *Rhinolophus ferrumequinum* (U95513) as outgroup) were performed with PAUP* 4.0b8 (Swofford, 2001). Resolution of internal nodes was evaluated using 10000 bootstrap replications with the PAUP* package.

HABITAT ANALYSIS

A Geographical Information System was set up with ESRI ArcView GIS version 3.2 (ESRI, 1999), using scanned 1:25000 paper maps from the Swiss Federal Office of Topography and Regione Lombardia CT10 1:10000 Technical Regional Digital Cartography as reference coverages. Land cover data was derived from the GEOSTAT data sets (Swiss Federal Statistical Office, 1997) for the Swiss portion of study area, and from CORINE Land Cover coverage (Commission of the European Communities, 1993) for the Italian part. Sampling sites were digitized heads-up using the above cited reference maps. Due to the different projection systems used in Switzerland and Italy, all the Italian geographic datasets were converted using ESRI

ArcGIS ARC/INFO 8.1 PROJECT routine (Booth, 1999) into Hotine Oblique projection (Swiss Reference Grid) and harmonised with GEOSTAT geodataset. Furthermore, CORINE Land Cover coverage has been converted to raster format and downsampled at the same spatial resolution of GEOSTAT, that is at a pixel size of 0.1 km^2. Because of different classification criteria in the two land cover datasets, classes were pooled and standardized according to Land Cover Classification System standards (Di Gregorio & Jansen, 2000), yielding the land cover classes shown in Table 1.

TABLE 1. The percentage of habitat use by the sibling species *Pipistrellus pipistrellus* (N = 3984 locations) and *P. pygmaeus* (N = 404 locations), and the percentage of available habitat determined for the entire study area based on 0.1 km^2 grid cells.

Habitat types	Habitat use		Available habitat
	P. pipistrellus	*P. pygmaeus*	
Woodland	46.4	46.0	44.5
Shrubland	0.4	0.5	3.9
Orchards, vineyards	6.3	7.2	5.3
Cultivated farmland	0.8	5.7	4.5
Meadows, alpine meadows	10.8	6.7	16.0
Water	4.2	9.9	5.8
Sterile land	0.6	0.5	7.7
Urbanised land	30.4	23.5	12.4

Finally, in order to obtain habitat class frequency for all the sampling sites, each point site was buffered using a fixed radius of 500 m, and converted into raster, and habitat class frequency was recorded as number of 0.1 km^2 pixels intersecting or contained in the buffered areas.

Habitat use and habitat overlap were both evaluated using a normalized version of the Proportional Similarity Index (PS_n), that is

$$PS_n = \frac{PS - \min(q_i)}{1 - \min(q_i)} \; ; [0 \leq PS_n \leq 1],$$

where PS is the Czekanowski's Proportional Similarity Index (Feinsinger *et al.*, 1981):

$$PS = 1 - \frac{1}{2} \sum_i |p_i - q_i| = \sum_i \min(p_i, q_i).$$

The symbols p_i and q_i indicate respectively the proportion of used and available land use class, when PS_n is used to estimate niche breadth, whereas the same notations indicate the proportion of habitat used by each species when PS_n is used to estimate niche overlap; $\min(q_i)$ represents the minimum observed resource item frequency.

Habitat preference by each species was evaluated using Ivlev's Electivity Index (Jacobs, 1974).

RESULTS

A total of 371 bats of different species were captured and analysed, 240 of which belonged to one of the two *Pipistrellus* sibling species. In a first step, distinction between *P. pipistrellus* and *P. pygmaeus* was based on acoustical data analysis. For a

subset of 18 individuals of each species, there was no difference in call duration (Mann-Whitney U-test: U = 105.5, p = 0.07), and in call maximum frequency (Fig. 2b, U = 121.0, p = 0.20). All other call parameters differed significantly between the two sibling species (Fig. 2, Mann-Whitney U-test all p < 0.01) and the largest difference was found for the frequency at maximum amplitude, on average 46.48 kHz for *P. pipistrellus* and 57.52 kHz for *P. pygmaeus* (Fig. 2e, U = 0, p < 0.01). Using only significant call frequency variables, a canonical discriminant function analysis (DFA) was calculated ($F_{approx.}$ = 35.0; df =5, 30; p < 0.001), which permitted us to determine 181 of the 201 recorded individuals' as *P. pipistrellus* and 20 as *P. pygmaeus*. The discriminant function, in its canonical form is

$$y = 21.101 + 0.033F_{start} - 0.071F_{min} - 0.407F_{maxint} - 0.081F_{end} + 0.102F_{1/2}$$

with a total discriminant capacity of 94%. Negative y values refer to *P. pygmaeus*, while positive ones refer to *P. pipistrellus* (Fig. 3).

Reconstruction of the phylogenetic tree by maximum parsimony and 10000 bootstrap replications showed that 33 of the 37 samples of *Pipistrellus*, belonged to the species *P. pipistrellus,* and four to *P. pygmaeus*. Of the four bats classified erroneously based on echolocation data, two genetically determined *P. pygmaeus* had been first determined as *P. pipistrellus* based on frequencies at maximum call intensity (F_{maxint}) of 45.40 and 45.94 kHz respectively, while two genetically determined *P. pipistrellus* had been first classified as *P. pygmaeus* based on (F_{maxint}) of 57.05 and 61.90 kHz.

Analysis of the 307 base pairs of the mtDNA cyt *b* gene confirmed DFA results, except for four animals that had been misclassified. The comparison of DNA sequences obtained with DNA reference sequences resulted in a K2P genetic distance of 0.4% between the two *P. pipistrellus,* 0.4% between the two *P. pygmaeus*, and 12.8 ± 0.5% between the two species. The MANOVA on the 12 morphological traits was statistically significant (F = 4.40; df = 12, 171; p < 0.001) and seven out of 12 morphological parameters differed significantly between the two sibling species at the 1% level (one-way ANOVA, level of significance p < 0.01, Table 2). A discriminant function for each species was calculated using only the four parameters that had the highest significance level (single one-way ANOVA, p < 0.001, Table 2). For *P. pipistrellus*, the slightly larger species, the discriminant function was described by

$$y = -499.91 + 20.58thumb + 3.93tibia + 2.24tail + 25.35\,forearm$$

while for *P. pygmaeus* it was

$$y = -463.34 + 18.66thumb + 3.02tibia + 1.84tail + 25.15\,forearm$$

The Jackknife procedure classified correctly 141 out of 175 (81%) *P. pipistrellus*, and 16 out of 20 (80%) *P. pygmaeus* (probability of uncorrect assessment: Wilk's lambda $F_{approx.}$ = 9.99; df = 4, 190; p < 0.0001). The canonical discriminant function obtained was described by

$$y = -24.92 + 1.28thumb + 0.61tibia + 0.26tail + 0.13\,forearm$$

with y < 0 indicating *P. pipistrellus* and y > 0 indicating *P. pygmaeus* (Fig. 5). Thus, taking into account that about 19% of animals were misclassified, morphological measurements alone are not sufficient to distinguish between the two pipistrelle sibling
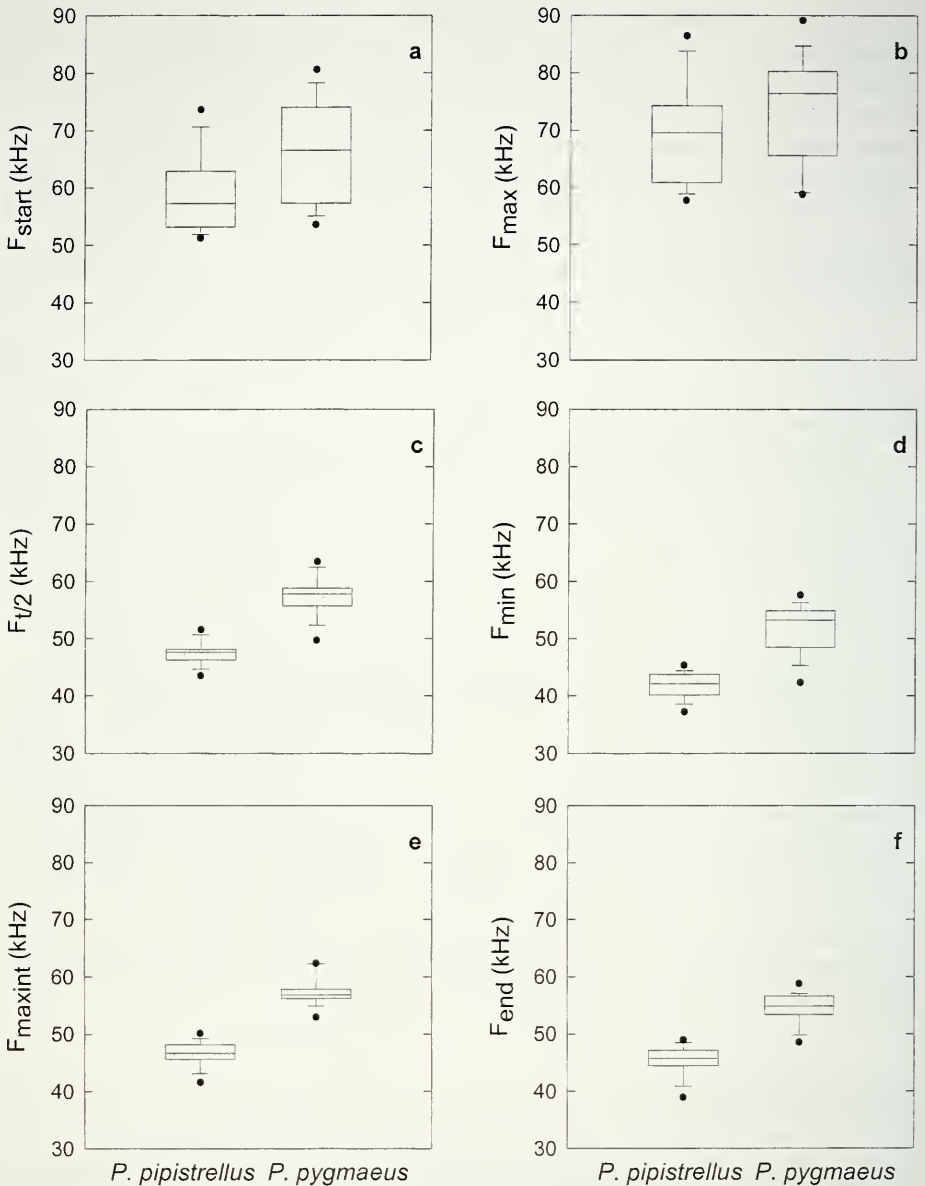
FIG. 2

Box-and-whisker plot comparison between *Pipistrellus pipistrellus* and *P. pygmaeus* ultrasonic search-phase call parameters: a: F_{start}, b: F_{max}, c: $F_{t/2}$, d: F_{min}, e: F_{maxint}, f: F_{end}.

species in our study area. Finally, following Häussler *et al.* (1999) we also calculated the mean differences between the second and third phalange of the 3rd finger, but found no significant difference between the sibling species (Mean difference ± sd in mm: *P. pipistrellus* 1.05 ± 0.70; *P. pygmaeus* 1.10 ± 0.91; t-test t = 0.27; df = 187; p = 0.79).
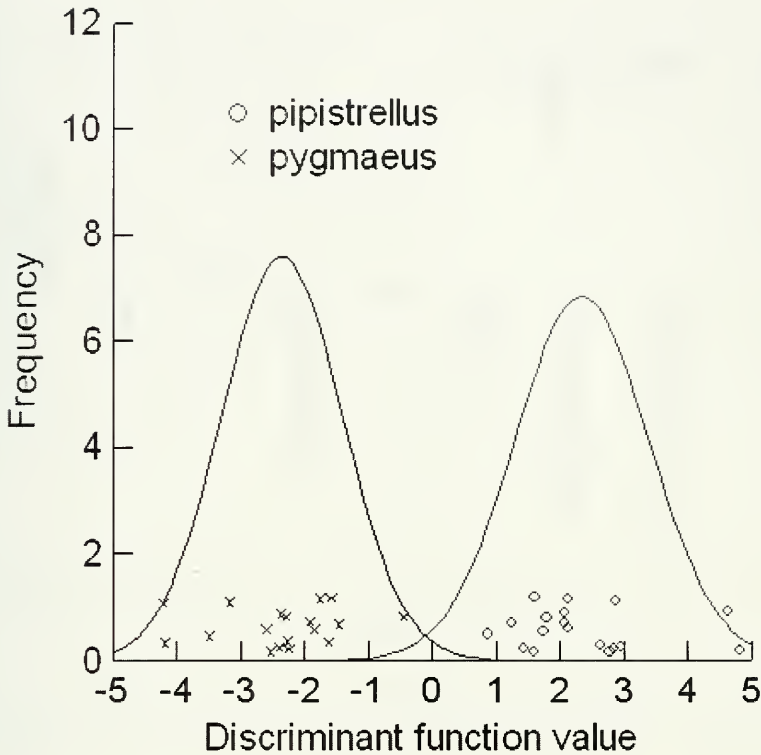
Fig. 3

Discriminant Function Analysis (DFA) canonical scores plot for ultrasonic search-phase call parameters in *Pipistrellus* spp.

Of the 33 *P. pipistrellus* examined, 23 (70%) had been captured at 10 different roosts in houses, eight (24%) along foraging sites in the Cantone Ticino, Switzerland, and two (6%) at one foraging site in the province of Como, Italy. Two *P. pygmaeus* had been captured at Gordevio, along the river Maggia, and two in bat boxes at Bosco Isolino, Cantone Ticino, Switzerland (Fig. 1).

Using both echolocation and genetic determination of the two sibling species, we compared the habitat characteristics of the sites where they had been found (N = 3984 for *P. pipistrellus*, N = 404 for *P. pygmaeus*, Table 1). Spatial niche breadth values were high and similar for both species (PS_n = 0.78 for *P. pipistrellus*; PS_n = 0.79 for *P. pygmaeus*), and there was a high degree of niche overlap (PS_n = 0.88). Each species used habitat types in a significantly different fashion if compared with habitat availability (Table 1; χ^2 = 41.4; df = 7; p < 0.001 for *P. pipistrellus*; = χ^2 = 29.2; df = 7; p < 0.001 for *P. pygmaeus*). The Ivlev's electivity index showed avoidance for sterile land (non-vegetated areas, bare rock and glaciers), shrubland and meadows, and positive selection for orchards and urbanised areas in both species. Farmland and water bodies were avoided by *P. pipistrellus*, but positively selected by *P. pygmaeus* (Fig. 4).
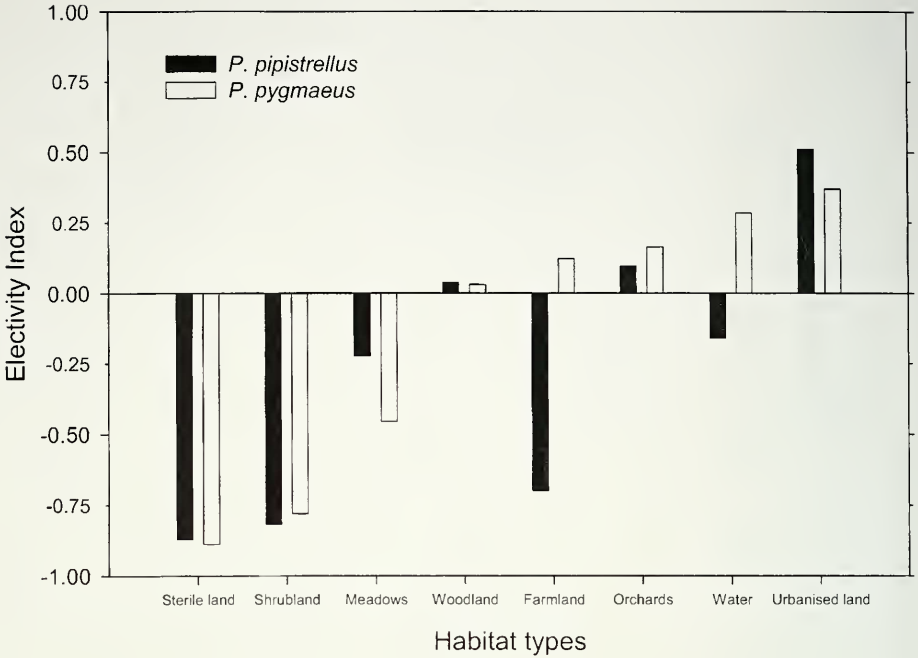
FIG. 4

Ivlev's Electivity Index values by habitat type for *Pipistrellus pipistrellus* and *P. pygmaeus*.

DISCUSSION

Morphological data from the two sibling species from the Rhine valley in Southwest Germany suggested that *P. pipistrellus* tends to be slightly larger than *P. pygmaeus* (Häussler *et al.*, 1999). In particular, there was a significant difference in mean length of the forearm, fifth finger and average tail length. This was confirmed by our data of external morphological measurements taken on live animals from alpine and prealpine areas of southern Switzerland and the provinces of Varese and Como, northern Italy. However, although differences in mean size existed, individual variation was large and there was considerable overlap in all measurements that were taken. Consequently, the best canonical discriminant function still misclassified about 20% of bats of each sibling species in our study area. Häussler *et al.* (1999) suggested that the difference in length between the 2nd osseous phalange and the terminal 3rd cartilaginous phalange of the 3rd finger, almost the same length in *P. pygmaeus*, while in *P. pipistrellus* the 3rd phalange is generally 2-3 mm shorter than the 2nd, is a useful diagnostic morphological characteristic that can be measured in the field. However, they do not produce any statistical evidence for this statement. Moreover, in our study area there was no such difference, indicating that it can not be used as a reliable diagnostic criteria. Thus, morphological measurements alone are not sufficient to investigate the presence/absence or distribution of the pipistrelle sibling species, at least in the southern Swiss Alps and confining areas in northern Italy.
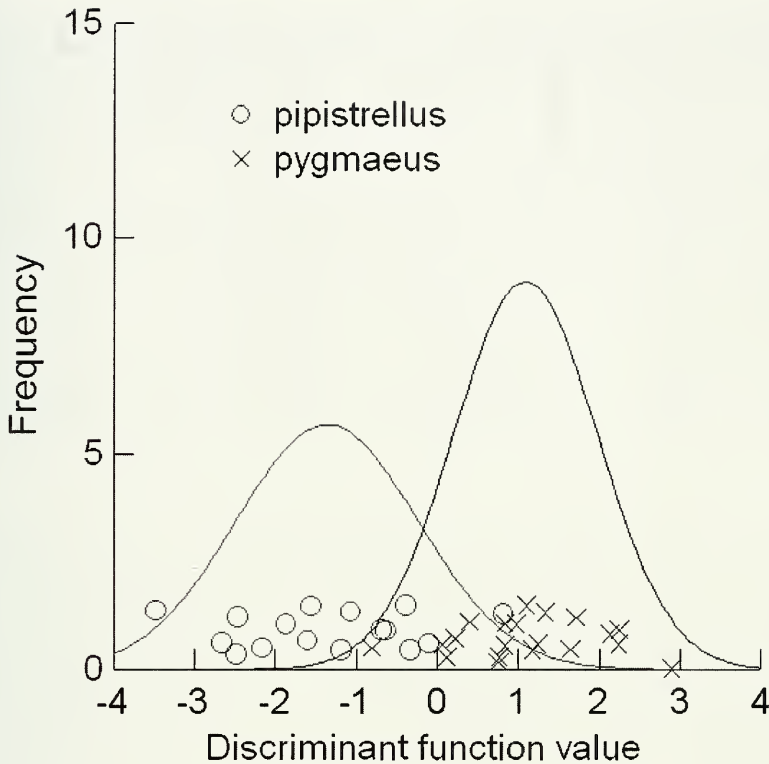
FIG. 5
Discriminant Function Analysis (DFA) canonical scores plot for biometric parameters in
Pipistrellus spp.

In contrast, a detailed analysis of echolocation calls allowed us to calculate a
canonical discriminant function which resulted in correct determination of 35 out of 36
bats (97%). In agreement with previous studies in different parts of Europe (Weid &
von Helversen, 1987; Jones & Van Parijs, 1993; Vaughan *et al.*, 1996, 1997; Barlow,
1997; Barlow & Jones, 1999; Russo & Jones, 2000; Mayer & von Helversen, 2001),
the frequency at maximum intensity of the search phase call was the call parameter
with the lowest inter-specific overlap (see Fig. 2). However, we must underline that
definitive species determination using the 307 bp sequence of the cyt-b gene of
mtDNA, demonstrated that in some cases, species classification based on echolocation
calls had failed. In part this might be caused by the individual variation in search phase
call frequency in both sibling species, found also in British colonies (Barlow & Jones,
1999), with some individuals using frequencies 5-7 kHz above or below the mean value
(see Fig. 2e). Other sources of erroneous classification can be linked with recording
conditions. Therefore, we want to stress that a low error rate when discriminating bat
species by means of their echolocation calls can only be ensured when the following
conditions are met: (1) high-quality equipment (bat detector and sonagraph or

computer-based Digital Signal Processing system); (2) ideal call recording conditions (no nearby obstacles, no interference by other bats with the recorded individual, limited distance between recorder and animal); (3) released bats in good condition, not stressed or suffering cold; (4) recording of the correct search phase call, not of other call types (e.g. social calls); (5) correct choice of call parameters for data analyses; and (6) appropriate statistical analysis.

Although other studies on sympatric pairs of cryptic bat species have shown there can be considerable habitat niche differentiation (Arlettaz, 1996; Arlettaz *et al.*, 1997), this was not the case for the sibling pipistrelle species in our study area which both had a large niche width, and a high degree, about 88%, of niche overlap. Nevertheless, some differences in habitat selection could be noted. As in south-west England, the foraging habitat of the soprano pipistrelle (*P. pygmaeus*) was more associated with water and riparian habitats, probably related to its diet consisting largely of insects with aquatic larval stages, while it clearly avoided urbanised areas (Barlow, 1997; Vaughan *et al.*, 1997). Also, in the landscape around maternity roosts, water courses and riparian habitat belts were present, as well as farmland, and no roosts were found in houses. This contrasted findings from Scotland, were *P. pygmaeus* selected house roosts that had a higher degree of nearby cover (large trees) than random houses (Jenkins *et al.*, 1998). However, these house roosts were surrounded by a greater area of deciduous and coniferous woodland than random buildings and nearly always within 500 m from a major river (Jenkins *et al.*, 1998), which underlines the importance of cover and the presence of nearby riparian habitats for this species (Oakeley & Jones, 1998). This was confirmed by our data with soprano pipistrelles roosting in nest boxes in deciduous woods, thus in a habitat with good cover, and foraging often along rivers and lakes. In contrast, most roosts of the common pipistrelle (*P. pipistrellus*) were in buildings. The latter species, commonly found in woodlands, also foraged intensively in cities and small villages as well as in nearby farmland. Although sometimes found at water or riparian foraging sites and roosts, it used these habitats less frequently than the soprano pipistrelle. Thus, habitat requirements are slightly different for the two sibling species (Vaughan *et al.*, 1997), with the common pipistrelle being a habitat generalist and the soprano pipistrelle selecting riparian habitats and farmland. However, overall, there were only small differences in our measurements of habitat use by the sibling species (see Table 1), resulting in a high degree of habitat niche overlap, and differentiation may be more accentuated for other niche components. In fact, both in our study and in others (Park *et al.*, 1996; Barlow, 1997; Oakeley & Jones, 1998; Jenkins *et al.*, 1998), there were no roosts occupied by both species simultaneously. Moreover, diet studies of the sibling pipistrelles in the British Isles showed that although both species ate mostly the dipteran suborder Nematocera and there was no difference between the phonic types in dietary breadth, there were some differences in food choice (Barlow, 1997). The main prey groups in the diet of *P. pipistrellus* were the families Psychodidae, Anisopidae and Muscidae, whereas the families Chironomidae and Ceratopogonidae occurred most frequently in the diet of *P. pygmaeus* (Barlow, 1997). Thus, stable co-existence of sympatric populations of *P. pipistrellus* and *P. pygmaeus* in heterogeneous landscapes seems possible because of differential selection of roost sites and preferred prey. Further studies are needed to test this hypothesis and to

TABLE 2. Morphological characteristics (mean ± sd) of 164 *Pipistrellus pipistrellus* and 20 *P. pygmaeus*. Determination of sibling species based on echolocation call frequency. Differences between means for each single parameter tested by one-way ANOVA (df = 1, 182 in all cases).

Parameter	*P. pipistrellus*	*P. pygmaeus*	F	p
Body mass (g)	4.95 ± 0.59	5.13 ± 0.92	1.22	0.27
Tumb (mm)	4.34 ± 0.36	4.02 ± 0.35	15.8	< 0.001
Tibia (mm)	11.74 ± 0.50	11.22 ± 0.67	17.8	< 0.001
Foot (mm)	6.53 ± 0.62	6.46 ± 0.36	0.15	0.70
Tail (mm)	31.98 ± 2.11	29.54 ± 1.84	25.1	< 0.001
Ear length (mm)	9.50 ± 0.88	9.36 ± 0.72	0.24	0.62
Forearm (mm)	31.19 ± 1.01	30.28 ± 1.29	14.1	< 0.001
III Finger (mm)	54.34 ± 2.56	52.27 ± 3.06	11.5	0.001
V Finger (mm)	40.00 ± 1.96	38.84 ± 1.87	6.37	0.012
III Phalanx (mm)	7.22 ± 0.53	6.82 ± 0.71	8.48	0.004
II Phalanx (mm)	8.27 ± 0.60	7.92 ± 0.58	6.59	0.011
Wing length (mm)	93.54 ± 3.39	91.26 ± 4.97	7.72	0.006

investigate whether the realised niche width of allopatric populations of *P. pygmaeus* differs from that of conspecifics in sympatry with the sibling species.

Collecting further information on habitat composition of foraging sites and location of preferred roosting sites of *P. pygmaeus* will be essential for landscape management and planning of specific conservation measures. For example, modification of certain riparian habitats and water courses by constructing sewage plants and conductors could cause marked changes of the invertebrate fauna of rivers, torrents and small lakes downstream of the sewage output, and thus alter food availability for, and activity of, foraging bats (see also Vaughan *et al.*, 1996). Since the soprano pipistrelle feeds primarily on insects with aquatic larval stages, such modifications might have serious consequences for the local survival of soprano pipistrelle populations.

We conclude that our genetic data definitively confirm that both *P. pipistrellus* and *P. pygmaeus* occur in Switzerland. Furthermore, our results indicate that correct monitoring of echolocation calls of pipistrelle bats is the most appropriate technique for large-scale studies on presence/absence and distribution of the two sibling species, but larger samples of genetically identified *P. pygmaeus* are needed to verify how reliable echolocation analyses are for correct species determination, as well as careful ultrasound recording.

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